

Title: Development of a versatile plant viral vector system for protein expression and virus induced gene silencing (VIGS)

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Abstract:

Plant virus-based vectors have been used for virus-induced gene silencing (VIGS) of plant gene expression and over expression of endogenous or exogenous genes. We have developed new vectors for VIGS and protein expression based on *Alternanthera mosaic virus* (AltMV), that can be introduced into plants by either mechanical inoculation of *in vitro* transcripts or via agroinfiltration. The vector includes both the *Cauliflower mosaic virus* (CaMV) 35S promoter and the bacteriophage T7 promoter, allowing both *in vivo* and *in vitro* transcription from the same construct. This construct alone was infectious by agroinfiltration despite the presence of 54 non-viral nucleotides between the 35S promoter and 5' end of the AltMV genome. Co-agroinfiltration of a construct expressing bacteriophage T7 RNA polymerase enhanced infectivity compared to *in vivo* transcription from the CaMV 35S promoter alone. Alternatively, *in vitro* transcripts from linearized AltMV binary vector can be delivered into plant cells by mechanical inoculation. An artificial bipartite viral vector delivery system was created by separating the AltMV replicase and [Triple gene block (TGB) + coat protein] coding regions into two constructs each bearing the AltMV 5' and 3' noncoding regions; the separate transcripts recombine precisely *in planta* to regenerate a full-length AltMV genome. The bipartite delivery system has the potential for high throughput cloning for gene discovery applications of VIGS. Substitution from L to P at position 88 of TGB1 in [TGB123-CP] affected RNA silencing suppression and alters the suitability of this versatile vector from protein expression to VIGS. A similar substitution in TGB1 of a *Potato virus X* infectious clone also altered efficiency of RNA silencing suppression. The nuclear/nucleolar localization of TGB1 is affected by this mutation, which will aid understanding of the mechanisms of RNA silencing suppression. AltMV has a wide host range including several *Nicotiana* species, tomato, eggplant, *Arabidopsis*, soybean, spinach, and diverse ornamental plants.